Available online at www.sciencedirect.com

SCIENCE DIRECT

Gynecologic Oncology 97 (2005) 612–617

Gynecologic Oncology

www.elsevier.com/locate/ygyno

Comparison of type-specific human papillomavirus data from self and clinician directed sampling

Susie Baldwin[a,1], Carlos Santos[f], Elena Mendez Brown[g], Thomas Nuño[d], Anna Giuliano[c], John Davis[b], Francisco Garcia[a,c,d,e,*]

[a]*The Department of Obstetrics and Gynecology, University of Arizona, Tucson, AZ 85724, USA*
[b]*Department of Pathology, University of Arizona, Tucson, AZ 85724, USA*
[c]*Arizona Cancer Center, University of Arizona, Tucson, AZ 85724, USA*
[d]*Arizona Hispanic Center of Excellence, University of Arizona, Tucson, AZ 85724, USA*
[e]*The Women's Center of Excellence, University of Arizona, Tucson, AZ 85724, USA*
[f]*The Departmento de Ginecologia, Instituto Nacional de Enfermedades Neoplasicas, Lima, Peru*
[g]*The Departamento de Citologia, Instituto de Seguridad Social al Servicio de los Trabajadores del Estado-ISSSTE, Hermosillo, Sonora, Mexico*

Received 8 December 2004
Available online 16 March 2005

Abstract

Objective(s). To compare the type-specific human papillomavirus (HPV) recovery from physician and patient-collected samples.

Methods. Three hundred thirty-four (334) women attending colposcopy clinics in three countries were enrolled in this cross-sectional study. Cervicovaginal samples were collected by patients and physicians and processed with polymerase chain reaction and reverse line blot genotyping. McNemar's Chi-squared tests and Kappa statistics were utilized to determine statistical associations between physician- versus patient-collected samples.

Results. Oncogenic HPV infection was identified in 23.2% of patient-collected specimens compared to 34.9% of physician-collected specimens. Physician sampling detected significantly more infections with type 16 and 52 than did self-sampling and significantly more oncogenic HPV infection overall. For non-oncogenic HPV detection, there was no statistical difference between physician- and patient-collected samples.

Conclusion(s). Patient sampling for HPV using a single vaginal brush does not identify all oncogenic HPV subtypes.

Keywords: Human papillomavirus; Cervical cancer; Self-sampling; Clinician directed sampling

Introduction

In the latter half of the twentieth century, morbidity and mortality due to cervical cancer decreased dramatically in the industrialized world secondary to widespread implementation of cytologic screening and treatment of pre-invasive cervical disease. However, cervical cancer continues to exact a large human and economic toll in developing countries, especially in Latin America, where it remains the second most common cause of cancer and cancer-related death among women [1,2]. Mexico and Peru are among the nations with the highest reported rates of cervical cancer in the world [1]. In the United States, where overall incidence of cervical cancer is low, the disease occurs disproportionately among Hispanic women. Hispanics in the US have twice the incidence of cervical cancer and a 40% higher mortality rate from cervical cancer compared to non-Hispanic whites [3,4].

Disparities in cervical cancer incidence across populations reflect generally low rates of cytologic screening. In

* Corresponding author. University of Arizona Health Sciences Center, 1501 N Campbell Avenue, P.O. Box 245078, Tucson, AZ 85724-5078, USA.
[1] Dr. Baldwin is now affiliated with the Veterans Affairs Ambulatory Care Center, Sepulveda, CA, USA.

0090-8258/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygyno.2005.02.001

Latin America, cytology-based cervical cancer screening programs have not achieved adequate coverage of the populations at greatest risk and have therefore been met with limited success [5–8]. Likewise in the US, Hispanic women have decreased access to health insurance and health care services, which limits their access to cervical cancer screening services [7–9]. Even when screening is available, lack of awareness, embarrassment, and fear of pelvic examination may create additional barriers, especially among poor women. It is this complex multi-factorial milieu that may account for Hispanic women in the US having among the lowest cytologic screening rates of any racial or ethnic group [9].

To overcome barriers associated with the need for pelvic examination, self-collection of vaginal samples for cytology and/or human papillomavirus (HPV) testing has been proposed as an alternative to physician-collected sampling. Studies in the US and Mexico have suggested that vaginal self-sampling is an acceptable approach among women [10,11]. The clinical validity of self-sampling, however, has not yet been well established, and it is unknown whether the vulvovaginal contamination inherent to the self-collected specimen leads to the detection of a different group of viral subtypes compared to clinician directed sampling, and what implications this may have on the performance characteristics of HPV testing for this indication. Additionally, most self-collection studies have not reported type-specific information about HPV detection in patient vs. physician-collected samples. Such information is important because tests for oncogenic HPV using self-collection modalities may hold particular promise as screening tools for underserved women. Furthermore, as prophylactic HPV vaccine development proceeds, knowledge of regional variations in viral types in different populations becomes essential.

In this analysis, we compare type-specific HPV infection in patient and physician-collected samples from women in seeking care in referral colposcopy clinics in Tucson (US), Hermosillo (Mexico), and Lima (Peru).

Materials and methods

Between January 1999 and June 2000, we performed a cross-sectional study of patient and physician sampling for cervical cytology and HPV. The University of Arizona Human Subjects Committee approved this study, as did the institutional authorities at the participating clinics in Peru and Mexico. We enrolled in the study a convenience sample of 334 women seeking care at three colposcopy clinics: the University of Arizona Health Sciences Center, Tucson, Arizona; el Instituto Nacional de Enfermedades Neoplasicas, Lima, Peru; and el Instituto de Seguro Social Trabajador Empleado, Hermosillo, Mexico. One hundred eight women enrolled in Tucson, 100 in Lima, and 126 in Hermosillo. Study methodology is described in detail and has been fully described elsewhere [12].

Eligible subjects were women 18 years or older, scheduled for colposcopic examination following abnormal screening or surveillance post therapeutic conization. Pregnant women, those with a history of hysterectomy, vaginal trauma, and/or vaginal laceration were excluded. Basic socio-demographic data including age and parity were also collected during the visit. All subjects provided informed written consent.

Participants were individually instructed on the collection of the vaginal specimen; patients inserted a soft endocervical collection brush (Cytobrush Plus, Medscand, Malmo Sweden) 5 to 6 cm into the vagina, rotated it five times, and placed it in a container filled with methanol buffer solution (PreservCyt Solution, Cytyc, Boxborough MA). All self-collections took place in a private examination room without the assistance of medical personnel.

Participants next underwent their scheduled gynecologic examination, which included visualization of the cervix through a speculum exam, assessment for vaginal trauma, and collection of cytology using a plastic Ayre spatula and endocervical brush. These collection devices were subsequently rinsed in the methanol buffer solution per the manufacturer's instructions (PreservCyt Solution, Cytyc, Boxborough MA). Colposcopists with a minimum of 6 years of experience conducted standard colposcopic examinations, performed directed cervical biopsies as indicated, and classified exams as negative, low-grade intraepithelial neoplasia, high-grade intraepithelial neoplasia, or carcinoma. While colposcopists in the US and Peru biopsied any visible lesions, Mexican colposcopists biopsied only lesions consistent with high-grade disease (suspected CIN 2/3 and carcinoma) in concordance with the local standard of care.

Research staff removed aliquots for HPV testing from liquid cytology specimens prior to the preparation of the cytology slides in order to minimize the theoretical possibility of cross contamination. HPV DNA analyses of exfoliated cervical cell samples were conducted using polymerase chain reaction (PCR) testing. Genomic DNA was extracted following standard techniques. Specimens were tested for the presence of HPV by amplifying 5 μl of the DNA extracts with the PGMY09/11 L1 consensus primer system [13] and AmpliTaq Gold polymerase (Perkin-Elmer, Foster City, CA). The samples were amplified using Perkin-Elmer GeneAmp PCR System 9700. HPV genotyping was conducted using the reverse line blot method [14] on all samples that were positive by PCR. This detection method utilized the HPV L1 consensus PCR products labeled with biotin to detect 27 HPV types. The HPV genotype strip contained 29 probe lines, detecting 27 individual HPV genotypes and two concentrations of the ß-globin control probe. Roche Molecular Systems, Inc. (Alameda, CA) provided all reagents. The Roche system detects oncogenic HPV types 16, 18, 26, 31, 33, 35, 39, 45,

51, 52, 55, 56, 58, 59, 68, 73, 82, and 83 and non-oncogenic types 6, 11, 40, 42, 53, 54, 57, 66, and 84.

Statistical analyses were performed using SAS System for Windows Version 8.1 (SAS Institute, Carey, North Carolina). McNemar's Chi-squared tests were conducted to determine whether type-specific association existed between patient-collected and physician-collected samples. Agreement between samples was measured with Kappa statistics. For all analyses a significance level was set at $P < 0.05$.

Results

Type-specific HPV results were available for 285 participants (85.3%). Forty two women with HPV detected in self-collected samples and 49 women with HPV in physician-collected samples had an unknown HPV type or had insufficient residual material to permit typing and therefore are not included in this analysis. Women from Peru were significantly less likely than women in the US and Mexico to have an unknown HPV type ($P < 0.001$ for both patient and physician-collected samples).

Table 1 summarizes the oncogenic and non-oncogenic HPV prevalence and type-specific prevalence by sampling method (patient-collected vs. physician-collected). Patient sampling detected significantly fewer oncogenic HPV infections than did physician sampling ($P < 0.001$); oncogenic HPV infection was identified in 23.2% of patient-collected specimens compared to 34.9% of physician-collected specimens. Agreement between patient-collected and physician-collected samples was fair; $\kappa = 0.45$.

Significantly fewer HPV 16 and 52 infections were detected in patient-collected samples compared to physician-collected samples ($P = 0.009$ for HPV 16; $P = 0.013$ for HPV 52). HPV 16 was the most commonly detected type, with a prevalence of 10.4% in patient-collected samples and 15.2% in physician-collected samples. HPV 52 was detected in only 1.19% of patient-collected samples but was the second most commonly identified type in physician-collected samples, with a prevalence of 3.88%. For all other oncogenic HPV types, there were no significant differences between patient and physician-collected samples. The next most prevalent oncogenic HPV types were HPV 31 and 45 in patient-collected samples and types 31 and 18 in physician-collected samples.

Non-oncogenic HPV prevalence was 5.01% in both patient and physician-collected samples, and there was no statistical difference between the collection methods. Agreement between patient-collected and physician-collected samples was very high at 97.6%, $\kappa = 0.738$. The most

Table 1
Type-specific HPV infection by collection method (patient vs. clinician)

Patient collection				Clinician collection			
HPV type	*n*	% of all types	% prevalence in population	HPV type	*n*	% of all types	% prevalence in population
Non-oncogenic				*Non-oncogenic*			
6	3	3.16	0.90	6	4	2.99	1.20
42	2	2.11	0.60	42	0	0.00	0.00
53	5	5.26	1.50	53	5	3.73	1.50
54	0	0.00	0.00	54	1	0.75	0.30
66	4	4.21	1.20	66	4	2.99	1.20
84	3	3.16	0.90	84	3	2.24	0.90
Oncogenic				*Oncogenic*			
16	35	36.84	10.48	16	51	38.06	15.27
18	4	4.21	1.20	18	7	5.22	2.10
26	0	0.00	0.00	26	1	0.75	0.30
31	6	6.32	1.80	31	10	7.46	2.99
33	1	1.05	0.30	33	0	0.00	0.00
35	2	2.11	0.60	35	3	2.24	0.90
39	2	2.11	0.60	39	2	1.49	0.60
45	5	5.26	1.50	45	6	4.48	1.80
51	4	4.21	1.20	51	6	4.48	1.80
52	4	4.21	1.20	52	13	9.70	3.89
55	4	4.21	1.20	55	1	0.75	0.30
56	2	2.11	0.60	56	2	1.49	0.60
58	3	3.16	0.90	58	6	4.48	1.80
59	2	2.11	0.60	56	2	1.49	0.60
68	1	1.05	0.30	68	0	0.00	0.00
73	0	0.00	0.00	73	2	1.49	0.60
82	1	1.05	0.30	82	1	0.75	0.30
83	2	2.11	0.60	83	3	2.24	0.90
Total	95				134		

prevalent non-oncogenic types in patient-collected samples were 53 and 55, while in physician-collected samples types 53, 66, and 6 were most prevalent.

Sixteen women had two or more HPV types detected through self-sampling while 20 had multiple types as detected by physician sampling (data not shown). While this difference was not statistically significant, concordance between the sampling methods for detecting multiple types was poor; kappa <0.01. Thirty six women, or 12.6% of the study population, were infected with at least 2 types of HPV. The most common combination of types detected was 16 and 45, present in 12.5% of patient-collected specimens and 10% of clinician obtained samples.

As shown in Table 2, HPV prevalence as detected by physician sampling varied significantly by country (P = 0.003). Self and clinician−collected specimens demonstrated nearly identical prevalence rates of non-oncogenic HPV infection (ranging from 4–6%). By comparison, oncogenic HPV prevalence was consistently highest among specimens from patients in Peru, 33% for self and 66% for clinician-collected specimens.

Additionally, among physician-collected samples, women with higher parity (3–10 births vs. 0–2) were more likely to have a positive test for non-oncogenic HPV type, although this was not the case for patient-collected samples. However, there was no association between age and type-specific HPV infection (data not shown).

Discussion

This study compares HPV types in patient-collected vaginal brush specimens with types detected in physician-collected cervical specimens processed from liquid cytology. The data we present here suggest that self-sampling using the technique we describe may not reliably identify all high-risk, oncogenic HPV types. While we found no significant difference between patient and physician-collected samples for most oncogenic types, HPV 16 and 52 were identified significantly less frequently in the self-sampled specimens. This is important because HPV 16 has been associated with as many as 58.9% of all cervical cancers worldwide [15]. Moreover, Peru has the highest reported prevalence of HPV 52 in cervical cancers [16]. A collection method that does not reliably detect these viral types may be of limited usefulness and represents a potential weakness of self-sampling, at least as performed in this study. Nearly 13% of women in our study population tested positive for multiple HPV types, consistent with previously published studies. This may be relevant since some investigators have reported that infection with multiple types is a risk factor for HPV persistence or for the development of cervical neoplasia.

Patient-collected samples were equivalent to physician-collected samples in the detection of non-oncogenic HPV types. One explanation for this finding is that non-oncogenic HPV strains may be more commonly found in the lower vagina or vulva than are oncogenic types, and their detection by self-collection methods may represent vulvovaginal contamination. Gravitt has in fact reported higher rates of non-oncogenic virus in patient compared to clinician−collected specimens [17]. Alternatively, cervical cells infected with non-oncogenic HPV may be more likely to be shed into the vagina than are cells infected with oncogenic HPV types. In any case, accurate detection of non-oncogenic HPV with self-sampling is not clinically useful in cervical cancer screening since these infections are benign and generally without clinical consequence. Of note, HPV 53 and 66, two non-oncogenic types, have been recently suggested to be “probably carcinogenic” by Munoz et al. [18] in their analysis of cervical cancers worldwide. However, re-categorization of these types into the oncogenic HPV group in our study did not affect the outcome of our analysis (data not shown).

Most published studies have found moderate or good correlation of HPV in patient-collected vaginal samples compared to physician-collected cervical swabs (kappa = 0.45–0.76) in screening [19,20] and referral populations [11,17,21]. The concordance of patient and clinician−collected specimens in our study (oncogenic HPV kappa = 0.45, non-oncogenic HPV kappa = 0.74) falls within the range described by other investigators. Discordant results were primarily attributable to differences in detection associated with the sampling approach. It is interesting to note however that, in several cases, HPV

Table 2
Prevalence of HPV infection by country and collection method

	Mexico *n* (% prevalence)	United States *n* (% prevalence)	Peru *n* (% prevalence)	Total
Patient collection				
Non-oncologic	5 (3.97)	6 (5.56)	6 (6.00)	17 (5.09)
Oncologic	24 (19.05)	27 (25.00)	27 (27.00)	78 (23.35)
Overall	29 (23.02)	33 (30.56)	33 (33.00)	95 (28.44)
Clinician collection				
Non-oncologic	5 (3.97)	6 (5.56)	6 (6.0)	17 (5.09)
Oncologic	34 (26.98)	23 (21.30)	60 (60.00)	117 (35.03)
Overall	39 (30.95)	29 (26.85)	66 (66.00)	134 (40.12)

was detected in the patient-collected specimen but not in the clinician collection. Five cases involved oncogenic (HPV types 33, 55, and 68) and two involved non-oncogenic (HPV type 42) viral types. Castle has postulated a vaginal tropism for certain phylogenetic groupings (A3/A4/A15) of HPV viruses [22]. Although only 3 cases fall into this group, the presence of a vulvovaginal rather than a cervical infection may explain these findings.

We have previously reported lower rates of recovery of oncogenic HPV types in patient-collected specimens compared to physician-collected specimens and lower sensitivity of patient-collected HPV specimens for diagnosing CIN 2/3 or invasive cancer among a referral population [12]. Similarly, Lorenzato et al. [23] reported a poor correlation of oncogenic HPV detection by self-sampling with HSIL and cervical cancer in a Brazilian screening population. Their group also found that, although there was no difference in overall HPV positivity between self-collected and physician-collected samples, the prevalence of high-risk types was significantly higher in physician-collected samples (26% vs. 17%) [23]. Another study by Palmisano et al. [24] also found significantly higher rates of high-risk HPV types, including specifically HPV 52, in cervical samples compared to vaginal swabs collected from 199 women. One explanation for this difference may have to do with the sampling technique. A recent report by Harper et al. demonstrated that the use of 2 sequential self-sampled swabs was equivalent to clinician-collected cervical swabs for the detection of oncogenic type HPV [11], while other investigators have achieved excellent concordance using a single Dacron swab [17].

An important limitation of this study is that it was conducted in a referral clinic with populations comprised of new incident cases of cervical disease as well as routine post therapeutic surveillance patients. Thus, our findings cannot be generalized to a screening population. Nonetheless, our study is the largest to date that evaluates HPV detection by type in patient-collected vs. physician-collected samples and as such represents an important contribution. Our type-specific results are consistent with previous prevalence studies [15,16,18,25], so we can infer that a successful prophylactic vaccine targeting types most commonly found in cervical cancer worldwide may have broad applicability to populations of women from northern Mexico, Peru, and the southwestern US.

Until such vaccines are available, however, screening for pre-invasive cervical disease remains our most important prevention strategy. With once-in-a-lifetime screening under consideration as a means of preventing cervical cancer in some resource-poor developing countries, the ability of a screening test to detect high-risk HPV types accurately becomes paramount. Our work suggests that a single brush self-sampling although safe [12] may not reliably replace physician-collected sampling for the detection of oncogenic HPV types. The conclusions we draw from our own experience can only be extrapolated to self-sampling using the same technique (soft endocervical brush inserted 5 to 6 cm into the vagina) and conducted in similar settings (colposcopy referral clinics). Self-collection may yet hold promise in some settings and may be particularly useful for the identification of negative patients for potential prophylactic HPV vaccination. Further work is required to optimize HPV detection in patient-collected specimens by improving sampling device and technique and/or alternatively by developing assays that are more appropriate for the type of material recovered in a self-collection setting.

Acknowledgments

The Population Council-Latin America Office provided seed funding for this study. Thin-layer cytology collection materials were provided at no cost by Cytyc Corporation (Boxborough, Massachusetts). This study was supported in part by grants from the National Institutes of Health and the Health Resource Services Administration (Garcia).

References

[1] Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2000: cancer incidence, mortality and prevalence worldwide. version 1.0. Lyon: IARC Press; 2001.
[2] Pisani P, Bray F, Parkin DM. Estimates of the world-wide prevalence of cancer for 25 sites in the adult population. Int J Cancer 2002; 97(1):72–81.
[3] Centers for Disease Control. Invasive cervical cancer among Hispanic and non-Hispanic women—United States. (2002); 51(47): 1067-70.
[4] O'Brien K, Cokkinides V, Jemal A, Cardinez CJ, Murray T, Samuels A, et al. Cancer statistics for Hispanics, 2003. CA Cancer J Clin 2003;53(4):208–26.
[5] Lazcano-Ponce EC, Moss S, Alonso de Ruiz P, Salmeron Castro J, Hernandez Avila M. Cervical cancer screening in developing countries: why is it ineffective? The case of Mexico. Arch Med Res 1999;30(3):240–50.
[6] Sankaranarayanan R, Budukh AM, Rajkumar R. Effective screening programmes for cervical cancer in low- and middle-income developing countries. Bull World Health Organ 2001;79(10):954–62.
[7] Medicine Io. Unequal treatment: confronting racial and ethnic disparities in health care. Washington DC: National Academics Press; 2003.
[8] Physicians ACo. Racial and ethnic disparities in health care. 2003:Position Paper.
[9] Society AC. Cancer facts and figures for Hispanic/Latinos 2003–2005. American Cancer Society; 2004.
[10] Dzuba IG, Diaz EY, Allen B, Leonard YF, Lazcano Ponce EC, Shah KV, et al. The acceptability of self-collected samples for HPV testing vs. the pap test as alternatives in cervical cancer screening. J Women's Health Gend -Based Med 2002;11(3):265–75.
[11] Harper DM, Noll WW, Belloni DR, Cole BF. Randomized clinical trial of PCR-determined human papillomavirus detection methods: self-sampling versus clinician-directed-biologic concordance and women's preferences. Am J Obstet Gynecol 2002;186(3):365–73.
[12] Garcia F, Barker B, Santos C, Brown EM, Nuno T, Giuliano A, et al. Cross-sectional study of patient- and physician-collected cervical cytology and human papillomavirus. Obstet Gynecol 2003;102(2): 266–72.

[13] Gravitt PE, Peyton CL, Alessi TQ, Wheeler CM, Coutlee F, Hildesheim A, et al. Improved amplification of genital human papillomaviruses. J Clin Microbiol 2000;38(1):357–61.
[14] Gravitt PE, Peyton CL, Apple RJ, Wheeler CM. Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. J Clin Microbiol 1998;36(10):3020–7.
[15] Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. J Natl Cancer Inst 1995;87(11):796–802.
[16] Santos C, Munoz N, Klug S, Almonte M, Guerrero I, Alvarez M, et al. HPV types and cofactors causing cervical cancer in Peru. Br J Cancer 2001;85(7):966–71.
[17] Gravitt PE, Lacey Jr JV, Brinton LA, Barnes WA, Kornegay JR, Greenberg MD, et al. Evaluation of self-collected cervicovaginal cell samples for human papillomavirus testing by polymerase chain reaction. Cancer Epidemiol, Biomarkers Prev 2001;10(2):95–100.
[18] Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348(6):518–27.
[19] Belinson JL, Qiao YL, Pretorius RG, Zhang WH, Rong SD, Huang MN, et al. Shanxi Province cervical cancer screening study II: self-sampling for high-risk human papillomavirus compared to direct sampling for human papillomavirus and liquid based cervical cytology. Int J Gynecol Cancer 2003;13(6):819–26.
[20] Wright Jr TC, Denny L, Kuhn L, Pollack A, Lorincz A. HPV DNA testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer. JAMA 2000;283(1):81–6.
[21] Sellors JW, Lorincz AT, Mahony JB, Mielzynska I, Lytwyn A, Roth P, et al. Comparison of self-collected vaginal, vulvar and urine samples with physician-collected cervical samples for human papillomavirus testing to detect high-grade squamous intraepithelial lesions. CMAJ 2000;163(5):513–8.
[22] Castle PE, Schiffman M, Bratti MC, Hildesheim A, Herrero R, Hutchinson ML, et al. A population-based study of vaginal human papillomavirus infection in hysterectomized women. J Infect Dis 2004;190(3):458–67.
[23] Lorenzato FR, Singer A, Ho L, Santos LC, Batista Rde L, Lubambo TM, et al. Human papillomavirus detection for cervical cancer prevention with polymerase chain reaction in self-collected samples. Am J Obstet Gynecol 2002;186(5):962–8.
[24] Palmisano ME, Gaffga AM, Daigle J, Brinkman J, Mire K, Lenczyk K, et al. Detection of human papillomavirus DNA in self-administered vaginal swabs as compared to cervical swabs. Int J STD AIDS 2003;14(8):560–7.
[25] Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189(1):12–9.